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SCREENING OF ANTIOXIDANT AND CYTOTOXIC COMPOUNDS EXTRACTED FROM *GYMNEMA SYLVESTRE R.Br.* LEAVES

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ABSTRACT

Plants are the carriers of written words, ideas and information and have been referred to as "the medium of thought". Medicinal plants were offer alternative remedies with tremendous opportunities. *Gymnema sylvestre* is a large, stout, much branched woody climber which grows predominantly in the tropical forest of central and south India. The purpose of this study was investigating experimentally about the antioxidant and cytotoxic potential of Gurmar. The bioactive compounds of the Gurmar were isolated and characterized by thin layer chromatography. The hexane, chloroform and methanol extracts were tested for antioxidant activity. The leaf extract of Gurmar plant have been proved to have good antioxidant activity which was undergone by *invitro* DPPH chemical assay and TLC spraying method. Methonal extract shows the best result than other two extracts. By using MTT assay, cytotoxicity was tested. Better activity was obtained in chloroform extract than other extracts in MTT assay.

Key words: *G. Sylvestre.*, DPPH assay, MTT assay.

Introduction

Higher plants are valuable sources of industrially important natural products, which include flavours, fragrances, essential oils, pigments, sweeteners, feed stocks, antimicrobials and pharmaceuticals[1]. Many compounds used in today's have a complex structure and synthesizing bioactive compounds[2]. Natural products and related drugs are used to treat 87% of all categorized human

diseases including bacterial infection, cancer and immunological disorders[3].

The World Health Organization has also recommended the evaluation of the plants effective in conditions were safe modern drugs are lacking. In recent years much attention has been devoted to natural antioxidant and their association with health benefits. Recently an intensive search for novel types of antioxidants has been carried out from numerous plant materials[4&5].

Antioxidants are added as redox systems possessing higher oxidative potential than the drug that they are designed to protect or as chain inhibitors of radical inducted decomposition[6]. Numerous studies on antioxidants present in plants have been conducted using the DPPH assay, including fruits and vegetables, medicinal plants, cereals and beans, spices and herbs, and teas and leaves[7-9].

Natural antioxidants have been known to can inhibit the formation of carcinogens from precursor substances, thus could provide preventive effect against cancer[10]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer[11].

Gymnema. Sylvestre (*G. Sylvestre*) commonly called as 'Gurmur' is widely distributed throughout India. The plant is known for its antidiabetic activity and rich in phytochemicals such as alkaloids, flavonoids, saponins, carbohydrates, and phenols[12].

Gymnema sylvestre leaves contain gymnemic acids, which are known to suppress transport of glucose from the intestine into the blood stream and a small protein, gurmar, that can interact with receptors on the tongue to decrease the sensation of sweetness in many foods[13].

G. Sylvestre leaf extract reduce hyperglycaemia in diabetic rabbits, rats and human and these glucose – decreasing effects may be medicated by increase in insulin secretion[14]. The plant is bitter astringent and useful in inflammations, dyspepsia, constipation, jaundice, haemorrhoids, cardiopathy, cough, asthma, bronchitis, intermittent, fever, amenorrhea, conjunctivitis and leukoderma. Roots are emetic and expectorant. This herb is a traditional remedy for snake bite [15].

In the present study, the selection of this plant for evaluation was based on its traditional usages.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used (various brands) were of analytical grade. DMSO, Quercitin, Proteinase k, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was

obtained from Sigma Aldrich Co., St. Louis, USA.

Plant material extraction:

Leaves of *Gymnema sylvestre* was collected from Tamil nadu agricultural university, Periyakulam campus in Tamil nadu. The leaves were washed, shade dried and powdered. The powdered leaves were extracted with hexane, chloroform, ethyl acetate and methanol using Soxhlet apparatus. After removal of solvents under reduced pressure, extracts were stored at -20ºc for further processing.

Antioxidant activity:

Leaf extract of *Gymnema sylvestre* were taken and subjected to antioxidant screening by chemical methods at different concentration.

DPPH assay:

The free radical scavenging property of extracts were analysed by 1, 2-diphenyl 1-picryl hydrazil (DPPH) assay. Hexane, chloroform, ethyl acetate and methanol extract were checked at different concentrations from 5-500µg were dissolved in 100µl methanol. To the methanol solution of 0.01mM DPPH, dissolved samples were added in separate test tubes and made up to 3 ml. An equal volume of DPPH solution and 0.1 ml methanol was taken as control. After 20 minutes, absorbance was recorded at 517 nm using UV spectrophotometer.

$$\%\text{inhibition} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) \times 100}{\text{Abs}_{\text{Control}}}$$

Invitro cytotoxic activity assay

Cell lines & culture medium:

Pherinal cancer cells were obtained. Cells were grown in Minimal Essential Medium (MEM) supplemented with 20% newborn calf serum. pH was maintained strictly between 7.4 - 7.7. cultures were maintained at 37ºc in 5% CO_2.

Cytotoxicity assay:

Cytotoxicity of sample on tumour cells was measured by microculture tetrazolium (MTT) assay. For the assays, 96-well microplates were seeded with 100µl medium containing 10, 000 cells in suspension. After 24 h incubation and attachment, the cells were treated with 6 fourfold dilution of crude extracts. Exactly from the stock solution (40 mg/ml), each extract sample was applied in a series of 6 dilutions (final concentrations ranging from 15.6 to 500 µg/ml) with a final DMSO concentration of 0.1% and was tested in quadruplicate. After 48 h incubation, cell viability was determined by adding (Sigma) tetrazolim salt as cytotoxicity indicator and by reading absorbance at 590 nm with a scanning multiwell spectrophotometer. Tetrazolium

salts are cleaved to formazan dye by cellular enzymes (only in the viable cells). The level of absorbance directly correlates to the metabolically active cells. Mitomycin C (~ 95 % HPLC, sigma-Aldrich) was used as a positive control.

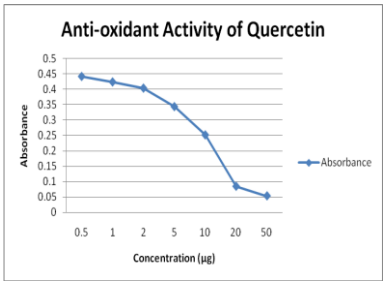
RESULTS

Extraction and Isolation of gurmar leaves:

Crude sample of hexane extract (3.2g), chloroform extract (5.8g), and methane extract (9.6g) were collected in separate vails and stored.

Antioxidant activity:

The antioxidant activity reflected by the DPPH radical scavenging assay was clearly observed in various solvent extracts of plant. Among the various solvent extracts of *G. sylvestre*, chloroform fractionated methanol fractions exhibited higher free radical scavenging activity.



Graph 1: Total antioxidant activity of G*ymnema sylvestre* leaves using DPPH scavenging method

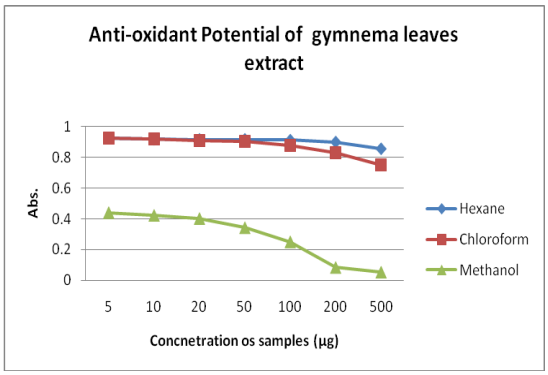
Lower EC value indicates greater antioxidant activity. Only 40 µg/ml of ethanol extract was required to reduce the DPPH radicals by 50% whereas the methane fraction needs 50µg/ml (Table 1).

Table 1: Total antioxidant activity of G*ymnema sylvestre* leaves using DPPH scavenging method

Sample (µg)	DPPH (ml)	Absorbance of samples at 517nm		
		Hexane	Chloroform	Methanol
5	2.9	0.923	0.923	0.441
10	2.9	0.916	0.916	0.423
20	2.9	0.915	0.907	0.403
50	2.9	0.915	0.901	0.343
100	2.9	0.910	0.875	0.251
250	2.9	0.898	0.829	0.084
500	2.9	0.855	0.749	0.053

This was significantly like the concentration needed for commercial antioxidant Quercetin. Refer graph 1. Methanol shows better activity (Refer graph 2) than other hexane & chloroform extracts.

Graph 2. Antioxidant potential of Gurmar compound against various extracts

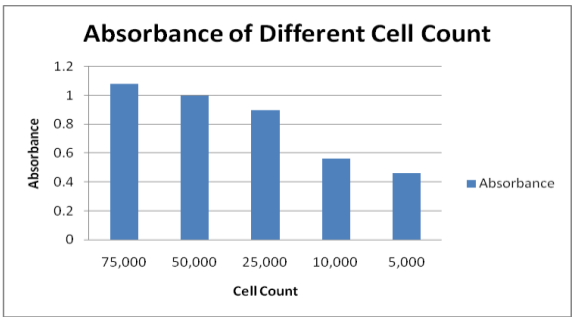


MTT assay with differential cell count:

Plate seeded with varying cell density was subjected to MTT assay. The result show proportional decrease in

absorbance and show the sensitivity and reliability of MTT assay (Graph 3).

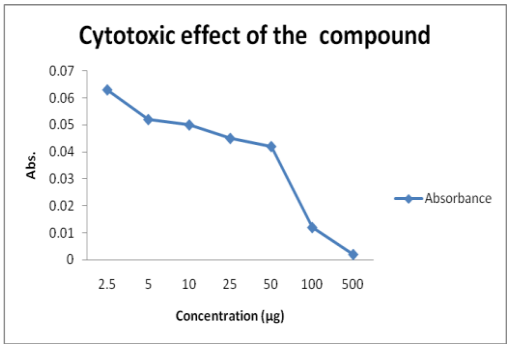
Graph 3. Absorbance of different cell count of sample



Cytotoxic potential:

Cytotoxicity of teak extracts were checked against fibroblast cultures. Different extracts showed high toxicity against both cell lines even at lowest concentration tested. Against chick embryo fibroblast cells, chloroform extract of leaves showed 87 % inhibition and hexane extract of leaf (54 %) in subsequent positions (graph 4).

Graph 4. The cytotoxic effect of chloroform extract of G. *sylvestre* on HCT-116 cells. The effect was measured by MTT cell viability assay.



Chloroform extract of leaves was able to exhibit high toxicity (95.3 %) against HEK293 cells also. Methanol extracts of leaves (73.2 %) were next in position showing toxicity against the later (Graph 5).

Graph 5. The cytotoxic effect of G. *sylvestre* leaf extract on HCT-116 cells. (MTT cell viability assay)

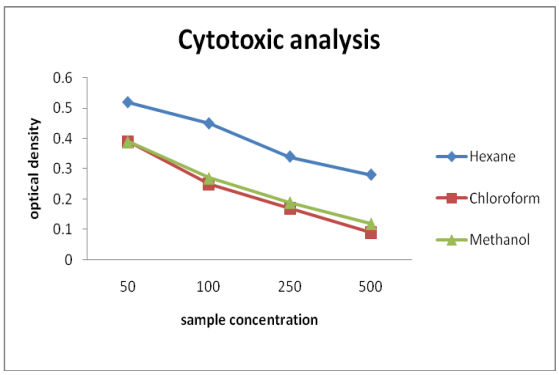
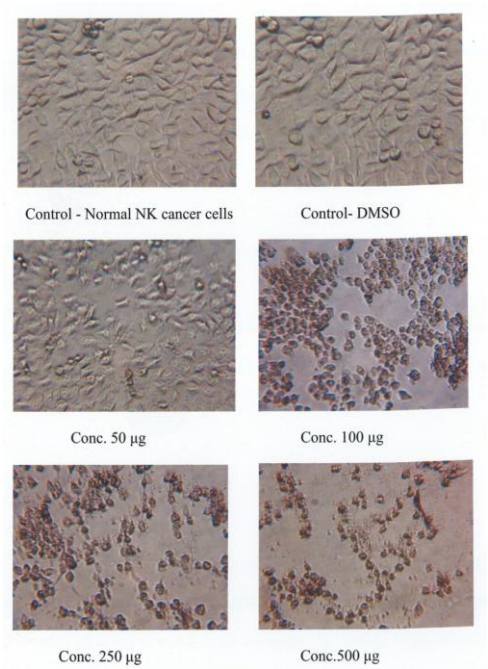


Plate 1: Morphological changes of cancer cell lines after plant extract treatment



DISCUSSION

Plant substances continue to serve as viable source of drugs for the world population and several plant-based drugs are in extensive clinical use. For the past few decades, number of plants has been widely used for the treatment of various diseases due to their antioxidant properties. Antioxidants can be defined as compounds that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction and by many other mechanisms and thus prevent disease[16].

CONCLUSION

This study confirms the importance and value of Gurmar on pharmacological basis is also highly notable along with its medicinal value. With the present study, we were able to highlight some of biological activities of *G. Sylvestre*. The in vitro antioxidant potential of crude methanolic extract of the *G. Sylvestre* showed good free radial scavenging activity depending on the concentration used.

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